

## WHAT IS CLAIMED IS:

1. A method for identifying an agent capable of inhibiting carbonic anhydrase activity of a CA IX polypeptide, said agent being selected from the group consisting of an antagonist anti-CA IX antibody, an antigen-binding fragment of said antagonist anti-CA IX antibody, a peptide, a peptoid, and a small organic molecule, said method comprising
  - a) combining an agent to be tested with a cell expressing said CA IX polypeptide under conditions suitable for detecting carbonic anhydrase activity; and
  - b) assessing the ability of said agent to inhibit said carbonic anhydrase activity, whereby inhibition of said carbonic anhydrase activity by said agent is indicative that said agent is an inhibitor.
- 15 2. The method of claim 1, wherein said agent is an antagonist anti-CA IX antibody or an antigen-binding fragment thereof.
- 20 3. The method of claim 2, wherein said anti-CA IX antibody or said antigen binding fragment thereof specifically reacts with an inhibitory epitope of said CA IX polypeptide to form an antibody-antigen complex, whereby the formation of said complex results in inhibition of carbonic anhydrase activity of said CA IX polypeptide.
- 25 4. The method of claim 3, wherein said inhibitory epitope is selected from the group consisting of an inhibitory epitope comprising contiguous amino acid residues of the carbonic anhydrase domain of said CA IX polypeptide, an inhibitory epitope comprising discontinuous amino acid residues of said carbonic anhydrase domain, and an inhibitory epitope comprising both contiguous and discontinuous amino acid residues of said carbonic anhydrase domain.
- 30 5. The method of claim 4, wherein said mammal is a human and wherein said CA IX polypeptide is human CA IX of SEQ ID NO:2, wherein said carbonic anhydrase domain is represented by amino acid residues 135 to 414 of SEQ ID NO:2.

6. The method of claim 5, wherein said inhibitory epitope comprises at least one amino acid residue selected from the group consisting of residues 226, 228, and 251 of SEQ ID NO:2.

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7. The method of claim 5, wherein said inhibitory epitope comprises at least 5 contiguous amino acid residues selected from the group consisting of residues 220 through 260 of SEQ ID NO:2.

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8. The method of claim 7, wherein said inhibitory epitope comprises at least one amino acid residue selected from the group consisting of residues 226, 228, and 251 of SEQ ID NO:2.

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9. The method of claim 3, wherein said anti-CA IX antibody is a human anti-CA IX monoclonal antibody or antigen-binding fragment thereof.

10. The method of claim 3, wherein said anti-CA IX antibody is a humanized anti-CA IX monoclonal antibody or antigen-binding fragment thereof.

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11. The method of claim 3, wherein said anti-CA IX antibody or antigen-binding fragment thereof further comprises a cytotoxin, a therapeutic agent, or a radioactive metal ion attached thereto.

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12. The method of claim 3, wherein said antigen-binding fragment is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

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13. A method for inhibiting proliferation of neoplastic cells in a mammal, where said neoplastic cells are characterized by expression of CA IX protein, said method comprising administering to said mammal a therapeutically effective dose of an agent that inhibits carbonic anhydrase activity of said CA IX protein, wherein said agent is identified by the method of claim 1.

14. The method of claim 13, wherein said agent that inhibits carbonic anhydrase activity of said CA IX protein is an antibody that is produced by a recombinant CHO cell or myeloma cell.

5 15. The method of claim 14, wherein said myeloma cell is an Sp2 or NS0 myeloma cell.

16. A method for identifying an agent that inhibits carbonic anhydrase activity of a CA IX polypeptide having a functional carbonic anhydrase domain, said 10 method comprising:

- a) combining an agent to be tested with a cell expressing said CA IX polypeptide under conditions suitable for detecting carbonic anhydrase activity; and
- b) assessing the ability of said agent to inhibit said carbonic 15 anhydrase activity, whereby inhibition of said carbonic anhydrase activity by said agent is indicative that said agent is an inhibitor.

17. The method of claim 16, wherein said CA IX polypeptide is selected from the group consisting of:

- a) human CA IX having the amino acid sequence set forth in SEQ ID NO:2;
  - b) the polypeptide set forth as residues 135-414 of SEQ ID NO:2;
  - c) a polypeptide having carbonic anhydrase activity, said 25 polypeptide comprising a fragment of human CA IX, said fragment comprising a functional carbonic anhydrase domain, wherein said carbonic anhydrase domain shares at least 70% sequence identity with residues 135-414 of SEQ ID NO:2; and
  - d) a polypeptide variant of human CA IX of SEQ ID NO:2, wherein said polypeptide variant shares at least 70% sequence identity with SEQ ID NO:2, said polypeptide variant having a functional carbonic anhydrase domain,
- 30 wherein said carbonic anhydrase domain shares at least 70% sequence identity with residues 135-414 of SEQ ID NO:2.

18. The method of claim 17, wherein said fragment comprises a functional carbonic anhydrase domain that shares at least 80% sequence identity with residues 135-414 of SEQ ID NO:2.

5 19. The method of claim 18, wherein said fragment comprises a functional carbonic anhydrase domain that shares at least 90% sequence identity with residues 135-414 of SEQ ID NO:2.

10 20. The method of claim 19, wherein said fragment comprises a functional carbonic anhydrase domain that shares at least 95% sequence identity with residues 135-414 of SEQ ID NO:2.

15 21. The method of claim 17, wherein said polypeptide variant shares at least 80% sequence identity with SEQ ID NO:2 and wherein said carbonic anhydrase domain shares at least 80% sequence identity with residues 135-414 of SEQ ID NO:2.

22. The method of claim 21, wherein said polypeptide variant shares at least 90% sequence identity with SEQ ID NO:2 and wherein said carbonic anhydrase domain shares at least 90% sequence identity with residues 135-414 of SEQ ID NO:2.

20 23. The method of claim 22, wherein said polypeptide variant shares at least 95% sequence identity with SEQ ID NO:2 and wherein said carbonic anhydrase domain shares at least 95% sequence identity with residues 135-414 of SEQ ID NO:2.

25 24. The method of claim 16, wherein said cell is a host cell selected from the group consisting of COS, Chinese hamster ovary, NIH-3T3, 293, and derivatives thereof.

30 25. The method of claim 16, wherein said cell is a cell line or a cell of a tissue that naturally expresses said CA IX.

26. The method of claim 16, wherein the ability of the agent to inhibit carbonic anhydrase activity is detected using a soft agar assay.

27. An inhibitor of carbonic anhydrase activity of a CA IX polypeptide that has been identified according to the method of claim 16, wherein said inhibitor is selected from the group consisting of a peptide, a peptoid, and a small organic  
5 molecule.

28. A method for inhibiting proliferation of neoplastic cells in a mammal, where said neoplastic cells are characterized by expression of CA IX protein, said method comprising administering to said mammal a therapeutically effective dose of  
10 an agent that inhibits carbonic anhydrase activity of said CA IX protein, wherein said agent is identified by the method of claim 16.

29. A composition comprising an agent that is capable of inhibiting carbonic anhydrase activity of a CA IX polypeptide, said agent being selected from  
15 the group consisting of an antagonist anti-CA IX antibody, an antigen-binding fragment of said antagonist anti-CA IX antibody, a peptide, a peptoid, and a small organic molecule, wherein said agent is identified by combining an agent to be tested with a cell expressing said CA IX polypeptide under conditions suitable for detecting said carbonic anhydrase activity; and assessing the ability of said agent to inhibit said  
20 carbonic anhydrase activity, whereby inhibition of said carbonic anhydrase activity by said agent is indicative that said agent is an inhibitor.

30. The composition of claim 29, wherein said composition is a  
pharmaceutical composition.  
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31. The composition of claim 30 further comprising a pharmaceutically acceptable carrier.

32. A method for inhibiting proliferation of neoplastic cells in a mammal, where said neoplastic cells are characterized by expression of CA IX protein, said method comprising administering to said mammal a therapeutically effective amount  
30 of the composition of claim 29.

33. A polypeptide consisting essentially of amino acid residues 135 to 414 of SEQ ID NO:2.

34. An improved method of treating a subject having a neoplasm that is 5 characterized by expression of CA IX protein, said method comprising administering a therapeutically effective amount of a pharmaceutical agent to said subject, wherein the improvement is that the pharmaceutical agent comprises an agent that inhibits carbonic anhydrase activity of said CA IX protein, wherein said agent is identified by the method of claim 1.

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35. The method of claim 34, wherein said agent is a monoclonal antibody.

36. A monoclonal antibody that inhibits carbonic anhydrase activity of 15 human CA IX or biologically active variant thereof that comprises a functional carbonic anhydrase domain, said functional carbonic anhydrase domain comprising a region of residues that is homologous to residues 229 to 256 of SEQ ID NO:2, wherein said antibody specifically reacts with an inhibitory epitope of said human CA IX or an inhibitory epitope of said biologically active variant thereof to form an antibody-antigen complex, whereby the formation of said complex results in 20 inhibition of said carbonic anhydrase activity, wherein said inhibitory epitope of said human CA IX comprises at least one of residues 229 to 256 of SEQ ID NO:2, and wherein said inhibitory epitope of said biologically active variant thereof comprises at least one residue of the region of residues that is homologous to residues 229 to 256 of SEQ ID NO:2.

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37. The monoclonal antibody of claim 36, wherein said inhibitory epitope of said human CA IX comprises at least two of residues 229 to 256 of SEQ ID NO:2 but less than 28 of residues 229 to 256 of SEQ ID NO:2.

30 38. A pharmaceutical composition comprising the antibody of claim 36 and a pharmaceutically acceptable carrier.

39. A method for assaying an antibody for the ability to inhibit carbonic anhydrase activity of a CA IX polypeptide, said method comprising

- a) combining an antibody to be tested with a cell expressing said CA IX polypeptide under conditions suitable for detecting carbonic anhydrase activity; and
- b) assessing the ability of said antibody to inhibit said carbonic anhydrase activity, whereby inhibition of said carbonic anhydrase activity by said antibody is indicative that said antibody is an inhibitor.

10 40. The method of claim 39, wherein said antibody is an existing antibody.